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Vitamin D and Inflammation in Major Depressive Disorder

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Abstract

Background: Increased inflammation is reported in Major Depressive Disorder (MDD), which may be more pronounced in suicidal subjects. Vitamin D deficiency may drive this pro-inflammatory state due to vitamin D's anti-inflammatory effects.

Methods: We quantified plasma 25-hydroxyvitamin D (25(OH)D) and inflammation markers interleukin (IL)-6 and tumor necrosis factor (TNF)-α, and other inflammatory indices, neutrophil-to-lymphocyte ratio (NLR) and white blood cell count (WBC) in 48 un-medicated MDD subjects (n=17 with mild-to-moderate suicidal ideation [SI]) and 54 controls. IL-6 and TNF-α were combined into a composite inflammation score.

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Author contributions

Cecile Grudet and Daniel Lindqvist formulated the research hypotheses; analyzed and interpreted the data; conducted literature searches; prepared tables/figures; and cowrote the manuscript. Owen Wolkowitz and Synthia Mellon designed the overall study; coordinated subject recruitment and blood sampling, and obtained funding. Owen Wolkowitz co-wrote the manuscript. Åsa Westrin, Victor Reus, Lena Brundin, Brenton Nier, Christina Hough, Johan Malm and Firdaus Dhabhar contributed to data interpretation and reviewed the manuscript for intellectual content. Johan Malm and Firdaus Dhabhar also assayed biomarkers. Victor Reus and Christina Hough were also involved in subject recruitment and data collection.

The authors declare no conflicts of interest

Results: There were no significant differences in 25(OH)D levels between MDD and controls (p=0.24) or between MDD with and without SI (p=0.61). However, 25(OH)D was negatively correlated with all measured inflammation markers; these correlations were stronger in MDD subjects, and particularly in those with SI. MDD status significantly moderated the relationships between 25(OH)D and NLR (p=0.03), and 25(OH)D and WBC (p<0.05), and SI significantly moderated the relationship between 25(OH)D and NLR (p=0.03).

Limitations: The study was cross-sectional, thereby limiting causal inference, and had a small sample size. Only seventeen of the MDD subjects had SI.

Conclusion: While 25(OH)D levels did not significantly differ in MDD vs. controls, or in MDD with or without SI, lower 25(OH)D was associated with indices of immune activation in MDD, especially in cases with SI. Although our findings do not address causality, they are consistent with findings that relatively low 25(OH)D levels in MDD are associated with a pro-inflammatory state.

Keywords

Vitamin D; 25(OH)D; Inflammation; Major Depressive Disorder; Suicidal Ideation

1. Introduction

Major Depressive Disorder (MDD) is a significant cause of morbidity and mortality worldwide, yet its pathophysiology and somatic manifestations are not fully understood. Increased blood inflammatory markers have frequently been reported in MDD subjects compared to healthy controls (Dhabhar et al., 2009; Dowlati et al., 2010; Howren et al., 2009; Miller and Raison, 2016; Schiepers et al., 2005), and this immune activation may be even more pronounced in suicidal individuals (Brundin et al., 2017; Brundin et al., 2015; Holmes et al., 2018; Kim et al., 2008; Tonelli et al., 2008). Meta-analyses and reviews indicate that MDD may be associated with decreased vitamin D levels (Anglin et al., 2013; (Humble, 2010), though individual study results have been mixed (Stefanowski et al., 2017). In addition to its well-recognized role in maintaining skeletal calcium balance, vitamin D exerts robust immunomodulatory effects in both the adaptive and the innate immune system by several different mechanisms. These can be summarized as favoring the T-helper 2 (Th-2) cytokine driven humoral immune response and decreasing the pro-inflammatory Th-1 cytokine driven cell-mediated immune response (van Etten and Mathieu, 2005). Several studies have examined plasma vitamin D levels in MDD (Anglin et al., 2013; Milaneschi et al., 2014; Parker et al., 2017; Shaffer et al., 2014; Stefanowski et al., 2017; von Kanel et al., 2015), but very few have investigated the relationship between vitamin D and inflammation in MDD (Accortt et al., 2016; Grudet et al., 2014).

We previously reported that plasma 25-hydroxyvitamin D (25(OH)D) levels are significantly lower in recent suicide attempters compared to controls and compared to depressed subjects without a recent suicide (Grudet et al., 2014). In that study, we reported a significant negative correlation between plasma 25(OH)D and pro-inflammatory markers in psychiatric patients, but not in controls (Grudet et al., 2014). This raises the possibility that a relative deficiency in 25(OH)D may promote a pro-inflammatory state and that the relationship

between 25(OH)D and inflammation may be differentially regulated amongst individuals with psychiatric symptoms and healthy subjects. Our previous study included psychiatric subjects with various diagnoses, and the majority had made a recent suicide attempt (Grudet et al., 2014). The suicidal subjects were recruited at the psychiatric emergency unit within one week after a suicide attempt. Furthermore, in that study, most --subjects took psychotropic medications and many had somatic co-morbidities, which could have impacted the results. In the present study, we aimed to replicate these findings of an inverse relationship between 25(OH)D and inflammation in a well characterized, medication-free and somatically healthy MDD sample and healthy controls. Based on our prior results (Grudet et al., 2014), we hypothesized that the negative association between 25(OH)D levels and inflammation would be more robust in MDD subjects compared to controls. Although we did not have the statistical power to directly compare MDD with suicidal ideation (SI) and MDD without SI, we also wanted to test, in exploratory analyses, if the negative correlation between 25(OH)D and inflammatory markers is especially pronounced in those with SI.

2. Subjects and methods

This study was approved by the Committee on Human Research of the University of California, San Francisco (UCFS) (protocol #10–00825). All patients gave their written informed consent before participating in the study and were compensated for their participation.

2.1.1. Study participants

Forty-eight un-medicated MDD subjects and 54 healthy controls were enrolled in the study. Demographic characteristics are described in Table 1. Subjects were recruited by flyers, bulletin board notices, Craigslist postings, newspaper ads, and clinical referrals in the San Francisco Bay Area between 2011–2015.

The Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID) (First, 1997) was used to determine all diagnoses, which were confirmed by a separate clinical interview with a board-certified psychiatrist. Control subjects had no history of any DSM-IV-TR Axis I disorder. Depressed subjects were diagnosed with current MDD, without psychotic features, and scored ≥17 on the 17-item version of the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960). Exclusion criteria for MDD subjects were: a suicide attempt or current suicidal intent within the past week, psychotic symptoms during their current major depressive episode, any history of psychosis that did not occur within the context of a past depressive episode, history of mania or hypomania, post-traumatic stress disorder (PTSD) or any eating disorder within one month of study participation, and substance/alcohol abuse or dependence within six months of study entry. Individuals who were clinically determined to have current suicide intent were excluded from the present study and referred to more appropriate resources for treatment. Aside from PTSD, the presence of co-morbid anxiety disorders was not exclusionary if MDD was considered the primary diagnosis.

All study participants underwent routine physical examination, medical history assessment, and routine blood screening (complete chemistry panel, including electrolytes, kidney and

liver function, protein and albumin, complete blood count with differential count, lipid panel, folate, fasting glucose, HbA1c, and thyroid function tests) to rule out the presence of any acute or chronic inflammatory disorders, neurological disorders, or other major medical conditions considered to be potentially confounding. None of the subjects had any vaccinations for at least six weeks prior to enrollment in the study. Further, all subjects were free of hormonal or psychotropic medications (e.g., antidepressants, steroids, hormonal birth control, etc.) or any other potentially interfering medications. None of the subjects were taking any vitamin supplements (including 25(OH)D) above the U.S. recommended daily allowances (25(OH)D, 15 μg/day) for six weeks prior to study start and all but one MDD participant stopped taking 25(OH)D entirely for two weeks before study start. If needed, MDD subjects were allowed to take short-acting sedative-hypnotics for sleep up to a maximum of three times per week, but none within one week of participation. On the day of each study visit, all subjects had to pass a urine toxicology screen (marijuana, cocaine, amphetamines, phencyclidine, opiates, methamphetamine, tricyclic antidepressants, and barbiturates) and a urine test for pregnancy in women of child-bearing potential.

2.1.2. Ratings—Depression severity was rated using the 17-item version of the Hamilton Depression Rating Scale (HDRS). Each HDRS rating session was conducted with two raters present who scored within one point of each other; if this was not achieved, a consensus rating was determined. For exploratory analyses, MDD subjects were categorized based on their HDRS suicidality item scores, which have a possible range of 0–4. Those with scores of 0 (indicating absence of suicidal ideation within the past week) were categorized as the "non-Suicidal Ideation group*"*(NSI; n=31). Those with scores of 1–3 (indicating responses ranging from "feelings that life is not worth living" to suicidal "ideas or gestures" in the past week) were categorized as the "Suicidal Ideation group" (SI; n=17). Subjects scoring 4 on the HDRS suicidality item, indicating a suicide attempt or current suicidal intent within the past week, were excluded from the study, as explained above and in (Khan et al., 2019). Physical activity was measured with the Yale Physical Activity Survey (YPAS) Vigorous Activity Index Score (Dipietro et al., 1993).

2.2. Study procedures

Subjects were admitted as outpatients to the UCSF Clinical and Translational Science Institute between 8:00 a.m. and 11:00 a.m., having fasted (except water) since 10:00 p.m. the night before. Subjects were instructed to sit quietly and relax for 25 – 45 minutes before blood samples were obtained. Following this blood draw, subjects underwent depression severity rating using the 17-item version of Hamilton Depression Rating Scale (HDRS).

2.3. Vitamin D assays, 25(OH)D_2 and 25(OH)D_3

Serum was collected into serum separator tubes and stored at −80° Celsius until assay. Stored serum samples were thawed once before the 25(OH)D_2 (vitamin D_2) and 25(OH)D_3 (vitamin D_3) analysis. The time of storage (4–7 years) is not likely to have had an impact on the quality of the analysis due to the relatively stable 25(OH)D molecule (Wielders and Wijnberg, 2009).

Analyses of $25(OH)D_2$ and $25(OH)D_3$ were done by liquid-chromatography-mass-spectrometry, model Sciex API 4000 LC/MS/MS (MA, USA). Coefficient of variation (CV) values were as follows: for $25(OH)D_2$, 6,0% at 40 nmol/L and 5% at 120 nmol/L and for $25(OH)D_3$ 6% at 40 nmol/L and 4% at 120 nmol/L. The lowest detection limit is 6 nmol/L for both for $25(OH)D_2$ and $25(OH)D_3$. The analyzes were conducted by The department of Clinical Chemistry at Scania University Hospital, which is accredited by SWEDAC (the Swedish Board for Accreditation and Conformity Assessment) and participates in the external assurance program of DEQAS (Vitamin D External Quality Assessment Scheme, UK). In line with clinical guidelines (Phinney et al., 2017), in cases of a $25(OH)D_2$ level >10 nmol/L, the $25(OH)D_2$ levels were added to the $25(OH)D_3$ levels in the statistical analysis, i.e. while describing vitamin D (25(OH)D) in the article, the sum of $25(OH)D_2$ and $25(OH)D_3$ is referred to as 25(OH)D. Only two MDD subjects had $25(OH)D_2$ levels >10 nmol/L; (11 nmol/L and 37 nmol/L, respectively. Blood sampling was performed across the year. Sampling season was divided into “Summertime”, which equals April-September and “Wintertime”, which equals October-March.

In this study, we used a cutoff of <50 nmol/L to indicate “lower vitamin D”. Levels between 50–74 nmol/L were considered “suboptimal/insufficient” and levels ≥75 nmol/L as “sufficient” (Holick, 2007).

2.4 Inflammatory markers assays

Plasma was collected into lavender EDTA vacutainer tubes and stored at −80° Celsius until assay. We assayed plasma cytokines IL-6 and TNF-α, two well established markers of inflammation that we previously reported to be elevated in MDD subjects in the current sample (Lindqvist et al., 2017). Absolute values of IL-6 and TNF-α are not reported here in order to avoid duplicate publication, but these can be found in our previous report (Lindqvist et al., 2017). In addition, we assessed the neutrophile-to-lymphocyte ratio (NLR), as well as white blood cell count (WBC), which are considered to be inflammatory markers of systemic, low-grade inflammation and which have recently been related to vitamin D (Akbas et al., 2016). Previous literature has suggested NLR to be an independent prognostic factor of morbidity and useful in stratification of mortality in several conditions, for example in certain cancers and cardiovascular diseases (Forget et al., 2017).

Assey methodology is described in Lindqvist et al (2017).

2.5 Statistics

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 25). All tests were 2-tailed with alpha = 0.05. 25(OH)D, NLR and WBC data were skewed; therefore we did a 90% winsorization to normalize the data. In order to limit the number of statistical comparisons, we decided to calculate a composite inflammation score by summarizing the z-scores of IL-6 and TNF-α. The neutrophil-to-lymphocyte ratio was based on the absolute neutrophil and lymphocyte count. For unadjusted group-wise comparisons, we used Student’s T-test and Pearson’s Chi-square test, ANCOVAs were used in order to adjust for potential confounders. For correlation analysis we used Pearson’s correlation and we used the PROCESS tool for SPSS in moderation analysis.

Based on their known association with vitamin D and/or inflammation we adjusted for the following variables in the analyses: age, sex, tobacco use, BMI and sampling season. In exploratory analyses, we subdivided MDD subjects into a "suicidal ideation group" (SI) and a "non-suicidal ideation group" (NSI), as defined in the "Ratings" section.

3. Results

3.1. Demographics and group comparisons

Demographic characteristics are summarized in Table 1. MDD subjects were more likely to be smokers (χ^2=8.9; p<0.01) and tended to have higher BMI (t=1.8; p=0.08), and lower YPAS score (t=1.8, p=0.07) than controls. There were no significant differences between MDD subjects and controls regarding age, sex, ethnicity, blood sampling season or physical activity (all p>0.60). 25(OH)D correlated negatively with BMI (r=−0.34; p<0.001) but not with YPAS score (r=0.07, p=0.48). Ethnicity was significantly associated with 25(OH)D levels (Caucasian mean 25(OH)D=59±20 nmol/L, Black/African-American mean 25(OH)D=35±10 nmol/L and all other mean 25(OH)D=45±19 nmol/L)(p<0.01). This pattern is consistent with known differences in 25(OH)D levels between ethnic groups due to differences in skin pigmentation (Weishaar et al., 2016). Women had significantly higher 25(OH)D levels compared to men (t=2.7; p<0.01) and current tobacco users tended to have lower 25(OH)D levels compared to tobacco non-users (t=1.9; p=0.07).

3.2. 25(OH)D and inflammatory markers in MDD and controls

Absolute levels of 25(OH)D and inflammation markers are summarized in Table 1. There were no significant differences in 25(OH)D, NLR or WBC between MDD subjects and controls (all p>0.6), or between MDD subjects with and without SI (all p>0.8). These results did not change after adjusting for age, sex, BMI, smoking and sampling season (all p>0.44).

Fifty-four percent of MDD subjects and 39% of controls had lower 25(OH)D (<50 nmol/L). Eight percent of MDD subjects and 17% of controls had sufficient 25(OH)D levels (>75 nmol/L) and 39 % of MDD subjects and 35 % of the controls had suboptimal 25(OH)D levels (50 – 74 nmol/L). None of these differences were significant (χ^2 = 2.97; p=0.28). There were no significant differences in categorical 25(OH)D levels between SI and NSI (χ^2 = 0.94; p=0.63).

3.3. Associations between 25(OH)D, inflammatory markers and symptom severity

Correlations between 25(OH)D and inflammatory markers are summarized in Table 2 and shown in Figures 1 and 2. 25(OH)D levels were significantly negatively correlated with the inflammation composite score across all subjects (r=−0.30, p<0.01) and within the MDD group alone (r=−0.34, p<0.05), but this relationship was not statistically significant within the healthy control group (r=−0.22, p=0.11). A significant negative correlation between 25(OH)D levels and the inflammation composite score was seen amongst MDD subjects with SI (r=−0.56, p<0.05), but not MDD NSI subjects (r=−0.20, p=0.30). A similar pattern was seen with the NLR and the WBC, which were both significantly negatively correlated with 25(OH)D levels in the MDD group (25(OH)D correlation with NLR r=−0.37 p=0.01, 25(OH)D correlation with WBC r=−0.37 p=0.01) but not in the healthy controls

(25(OH)D correlation with NLR r=0.08 p=0.57, 25(OH)D correlation with WBC r=0.00, p=0.99). Within the MDD group, this relationship was significant in those with SI (25(OH)D correlation with NLR r=−0.71 p<0.01, 25(OH)D correlation with WBC r=−0.52 p<0.05) but not in the NSI group (25(OH)D correlation with NLR r=−0.16 p=0.39, 25(OH)D correlation with WBC r=−0.26 p=0.16).

MDD status was a significant moderator of the relationship between 25(OH)D and NLR (p=0.03; Fig. 1a) and between 25(OH)D and WBC (p<0.05; Fig. 1b), but not between 25(OH)D and the inflammation composite score (p=0.31; Fig. 1c). Adjusting for age, sex, BMI, sampling season and smoking did not significantly change the results of these moderation analyses.

In MDD subjects only, presence of SI was a significant moderator of the relationship between 25(OH)D and NLR (p=0.03), but not WBC (p=0.17) or the inflammation composite score (p=0.18). Adjusting for age, sex, BMI, sampling season and smoking did not significantly change the results of these analyses.

Total HDRS scores were not significantly correlated with 25(OH)D, NLR, WBC or inflammation composite score (all p>0.15).

4. Discussion

Though we did not detect any significant group differences in 25(OH)D levels between MDD subjects and healthy controls, nor any significant association between 25(OH)D and depression severity, we did find that the relationship between 25(OH)D and inflammation differed between MDD and control subjects. Specifically, 25(OH)D levels were significantly and negatively correlated with inflammatory markers in MDD, but not in healthy controls. Exploratory analyses found no significant group differences in 25(OH)D levels between MDD subjects with and without SI, though the relationship between 25(OH)D and inflammation also differed between these two groups. Specifically, 25(OH)D levels were significantly negatively correlated with inflammatory markers in MDD subjects with SI, but not in those without SI. Adjusting for for age, sex, BMI, sampling season and tobacco use did not change these results.

In the present study, we did not find a significant difference in 25(OH)D levels between MDD subjects and healthy controls, nor did we find a correlation with depressive symptom severity, which is in line with some previous reports (Dana-Alamdari et al., 2015; Grudet et al., 2014), but not others (Hoogendijk et al., 2008). There are several potential explanations for these inconsistent findings across studies, including differences in various sample characteristics such as sample size, age, gender, BMI, ethnicity, depression severity, degree of comorbidity, lack of data about UVB-radiation exposure, seasonality and latitude, medication status (especially antidepressants medication and vitamin D or multi-vitamin intake), smoking habits and dietary habits. Results from several previous studies suggest that vitamin D levels are the lowest among subjects with severe-to-very severe depression symptoms. For example, in the largest cross-sectional study to date (the Netherlands Study of Depression and Anxiety, NESDA, comprising > 2000 subjects) participants with

current depressive disorder had significantly lower vitamin D levels compared to controls (Milaneschi et al., 2014). The vitamin D levels declined progressively in a “dose-response” manner in relation to depression severity. Thus, the difference in vitamin D levels between MDD subjects and healthy controls was consequently more pronounced in those with the most severe symptoms. In the present study, most of the MDD subjects had only moderate depression severity. Moreover, in the study by Milaneschi et al., a large proportion of the subjects used antidepressants and had somatic co-morbidities, while all the subjects in the present study were un-medicated and somatically healthy. There are several additional factors that may explain why we did not find any group difference in 25(OH)D levels between MDD subjects and controls. Although we did adjust for several known vitamin D-related factors such as sex, age, BMI, smoking and seasonality, we cannot rule out the possibility of residual confounding due to factors that we were not able to take into account, such as dietary habits and actual UVB-radiation exposure. Lastly, low vitamin D may be more closely associated with certain clinical features of MDD than others. For instance, we and others have previously shown that low 25(OH)D may be associated with attempted and completed suicide (Grudet et al., 2014; Umhau et al., 2013), suggesting that vitamin D may be more closely linked suicidal depression rather than a MDD diagnosis *per se* (Tariq et al., 2011). In the current study we were not able to confirm (or refute) this hypothesis since a suicide attempt or current suicidal intent within the past week were exclusion criteria. Consequently, the lack of difference in 25(OH)D levels between MDD with and without suicidal ideation does not contradict our previous results where recent suicide attempters had significantly lower 25(OH)D levels than MDD without a recent suicide attempt, and healthy controls (Grudet et al., 2014).

In the present study, we found a significant and negative correlation between 25(OH)D and the inflammatory composite score, based on IL-6 and TNF-α levels, in MDD subjects but not in the controls. Additional markers of systemic and low-grade inflammation, NLR and WBC, were also significantly negatively correlated with 25(OH)D in MDD subjects, but not in controls. We cannot rule out that the lack of significant findings in the controls is a result of insufficient power, although the sample size of the MDD group was smaller than that of the control group. While dividing MDD subjects according to presence/absence of suicidal ideation (SI or NSI), these correlations lost significance in the NSI group but remained significant in the SI group. These results should be interpreted with caution, however, due to the small subgroups of MDD subjects with (n=17) and without SI (n=31). These data are largely consistent with results from our previous study, where a significant negative association between 25(OH)D and inflammatory markers was found in MDD outpatients without a recent suicide attempt and in inpatients with mixed psychiatric diagnoses who had a recent suicide attempt, but not in healthy controls (Grudet et al., 2014). Only a few studies to date have investigated the relationship between vitamin D and inflammation in psychiatric cohorts (Accortt et al., 2016; Antai-Otong, 2014; Grudet et al., 2014). However, one prospective study also provides evidence of an inverse relationship between vitamin D and inflammation in depression. This study reported an inverse correlation between vitamin D levels and post-partum depression (PPD), and this effect was significantly moderated by inflammatory markers, i.e. low vitamin D was associated with PPD, but only in those women with higher inflammation (Accortt et al., 2016). It is not known whether the

association between vitamin D and inflammation in subjects with MDD speaks to a causal relationship. Whereas it is usually presumed that low vitamin D levels may promote an inflammatory state (as described in Introduction), it is also possible that higher inflammation can lead to lower vitamin D levels. In fact, some studies suggest that an inflammatory process in the body *per se* is causing a decline in vitamin D levels (Autier et al., 2014; Mangin et al., 2014). This theory proposes that the circulating, inactive form of vitamin D decreases in response to an immunological challenge, i.e. the vitamin D that we measure is being consumed, which would then reflect the negative association between 25(OH)D and inflammatory markers observed in our sample (Albert et al., 2009). As such, it is possible that low vitamin D may promote low-grade inflammation, inflammation may decrease vitamin D, or that this relationship may be bidirectional.

Vitamin D's regulation of systemic immune functions involves key regulatory mechanisms of both innate and adaptive immune responses. These processes are mainly mediated by influencing downstream effects of activated Toll-like receptors (TLRs), expressed on antigen-presenting cells such as macrophages and dendritic cells (DC). TLRs are key players in both the innate and the adaptive immune systems and excessive activation of these induce chronic low-grade inflammation (Hemmi and Akira, 2005), associated with various pathological conditions such as depression and suicide (Brundin et al., 2017; Schiepers et al., 2005). There is an interplay between TLRs and vitamin D and many studies have demonstrated a negative correlation between the expression of TLRs and 25(OH)D, especially in diseases with an inflammatory component (Adamczak, 2017). By influencing the DC phenotype and by inhibiting the maturation of DCs, vitamin D has modulatory effects on the nature of the T cell response upon TLR activation (Chambers and Hawrylowicz, 2011). Vitamin D inhibits or suppresses, directly or indirectly, the proliferation and differentiation of T helper cells into pro-inflammatory Th-1 and Th-17 immune cells. Vitamin D also promotes the production of anti-inflammatory Th-2 and regulatory T immune cells (Tregs), the latter being an important inhibitor of many immune responses and a maintainer of the immunologic tolerance in the periphery (Chambers and Hawrylowicz, 2011; Do et al., 2008; Sassi et al., 2018). The suggested net result of the effects of Vitamin D is a skew towards a Th-2/Treg immune response with increased production of anti-inflammatory cytokines such as IL-10, IL-4, IL-5 and transforming growth factor (TGF)-β, and decreased pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, interferon (INF)-γ, TNF-α and IL-12 (Aranow, 2011; Baeke et al., 2010; Umar et al., 2018; Zhang et al., 2012).

In addition to its influence on systemic inflammation, Vitamin D may also directly influence immune functions of the brain. The 25(OH)D activating enzyme, 1α-hydroxylase, and vitamin D receptors (VDRs) are present is numerous cell types and tissues in the body, including most cells of the immune system (Skrobot et al., 2018) as well as in neurons and glial cells, thus making it possible for vitamin D to act locally in the brain via autocrine, paracrine and intracrine mechanisms (Borges et al., 2011; Cannell et al., 2014). In animal studies, activated microglia have been demonstrated to increase the expression of VDR and 1α-hydroxylase, thus enhancing their sensitivity to vitamin D. (Hanisch, 2002). Notably, activated microglia exposed to 25(OH)D and $1,25(OH)_2D$ had reduced expression of pro-inflammatory cytokines IL-6, IL-12, and TNF-α and increased expression of the

anti-inflammatory cytokine IL-10 (Boontanrart et al., 2016; Singhal and Baune, 2017). Activated microglia cells exposed to vitamin D may therefore promote a switch from the pro-inflammatory Th-1 cell immune response to the less inflammatory Th-2 cell immune response in the brain. Thus, by altering the immune response by microglia, vitamin D is suggested to be a control mechanism for avoiding a prolonged inflammatory state in the CNS (Singhal and Baune, 2017).

Some studies have reported that MDD subjects are at a higher risk of vitamin D deficiency and that vitamin D supplementation may reduce depressive symptoms, particularly among those with severe deficiency and clinically significant depression (Anglin et al., 2013; Bahrami et al., 2018; Ju et al., 2013; Kjaergaard et al., 2011; McCue et al., 2012; Milaneschi et al., 2014; Parker et al., 2017; Shaffer et al., 2014; Stefanowski et al., 2017). However, randomized controlled studies investigating vitamin D treatment in depression have yielded inconsistent results. In a meta-analysis, Gowda et al. (2015) did not find any significant reduction in depression after vitamin D treatment. Most previous studies, however, focused on individuals with low levels of depression and with sufficient vitamin D levels at baseline, which may be two reasons why any beneficial effects of vitamin D treatment could not been detected. (Gowda et al., 2015; Kjaergaard et al., 2012).

A strength of the present study is the inclusion of medication-free, well phenotyped, somatically healthy MDD subjects as well as healthy controls, where none had taken any vitamin D supplements above the recommended daily dose for at least six weeks before study start. All subjects were free of any psychotropic medications (including antidepressants) and other potentially interfering medications for a minimum of six weeks. A relevant limitation of the study is the cross-sectional study design, which makes it impossible to investigate any causality in the material. Additionally, due to the limited sample size, we were unable to perform subgroup analyses in 25(OH)D deficient/sub-optimal/sufficient subgroups. Further, the sample sizes of the SI and NSI MDD subgroups were too small for meaningful conclusions to be drawn, but they do suggest hypotheses to be tested in future studies.

5. Conclusions

In summary, we here report a significant negative relationship between 25(OH)D and inflammatory markers in MDD subjects but not in controls, and this association was strongest in those MDD subjects with SI. Our findings suggest that low 25(OH)D is associated with a pro-inflammatory state, that is frequently observed in depressed and suicidal individuals, and that this relationship between 25(OH)D and inflammation may be differentially regulated in MDD subjects (especially in those with SI) and healthy controls. Although speculative and a question for future research; there is a possibility that vitamin D supplementation might attenuate inflammation in MDD in individuals with low vitamin D levels, which would be of importance since inflammation may underlie certain medical comorbidities in MDD and may foster antidepressant resistance.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Highlights

- Vitamin D was negatively correlated with inflammation in MDD but not in controls
- These associations were stronger in those MDD subjects with suicidal ideation
- Relatively low vitamin D may promote inflammation in depressed and suicidal subjects

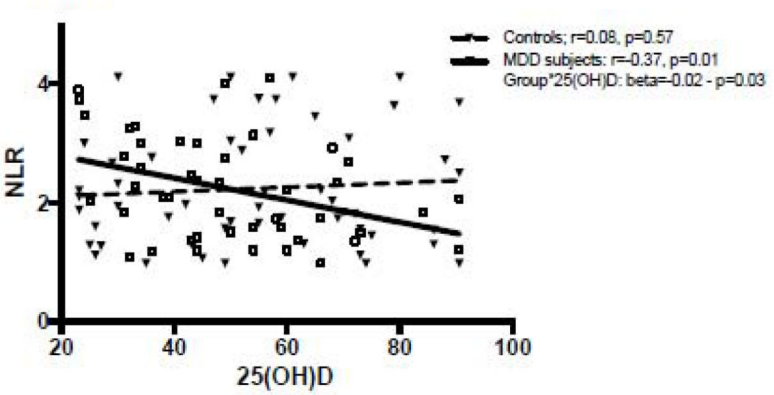

Figure 1B

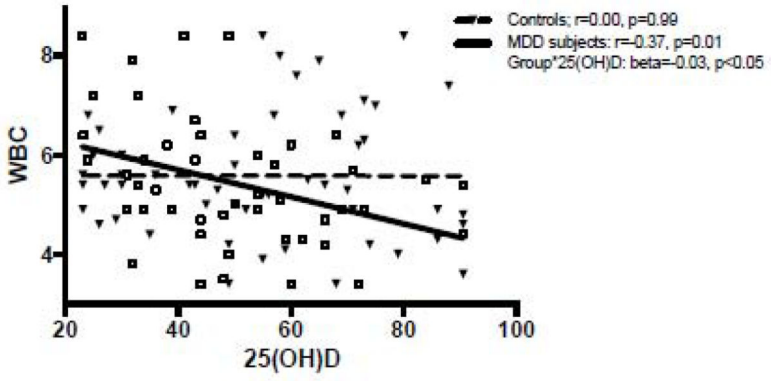

Figure 1C

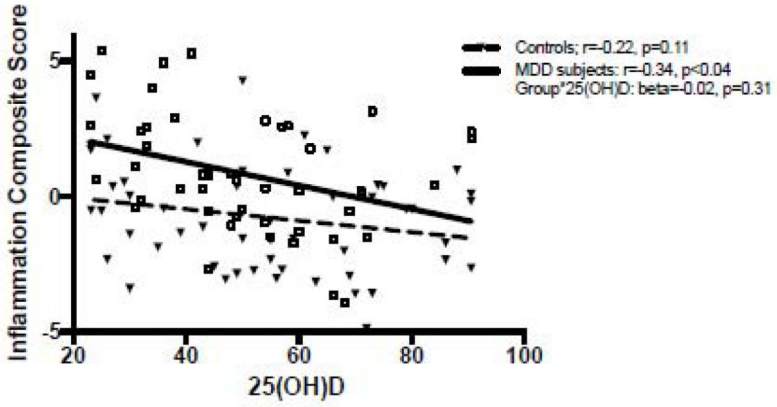

Figure 1.

A-C. Inflammation markers plotted against 25(OH)D in MDD subjects and healthy controls. Data were normalized using 90% winsorization.

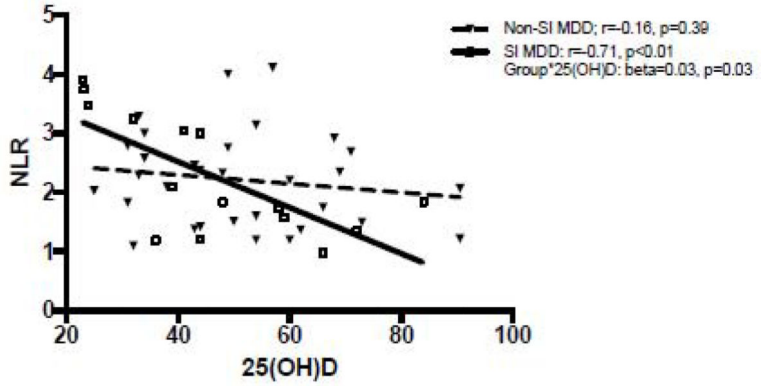

Figure 2B

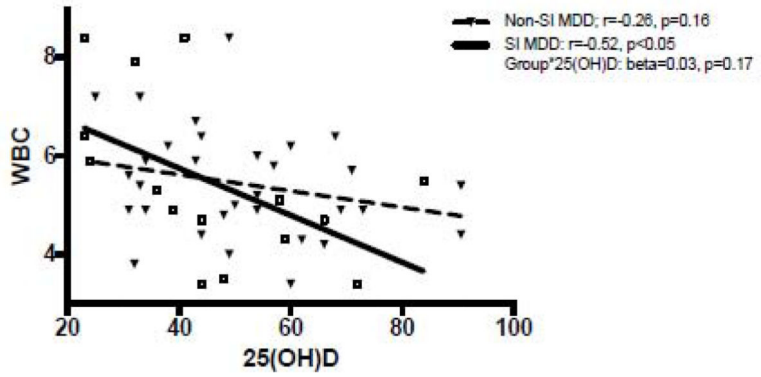

Figure 2C

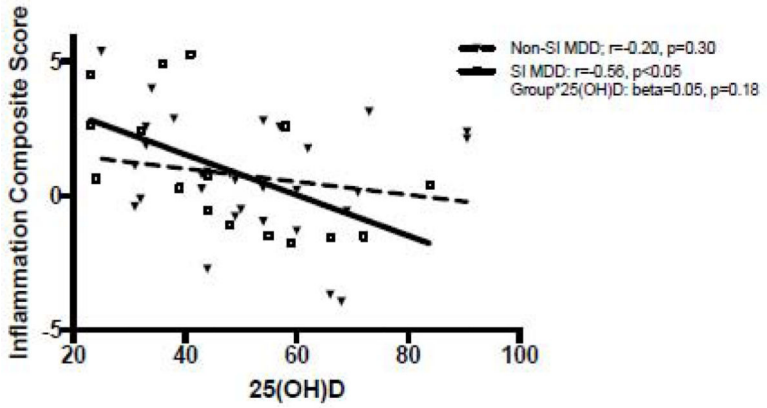

Figure 2.

A-C. Inflammation markers plotted against 25(OH)D in MDD subjects with suicidal ideation (SI) and MDD subjects without suicidal ideation (NSI). Data was normalized using 90% winsorization.

Table 1.

Demographic characteristics of all subjects and MDD subdivided into Suicidal Ideation/Non-Suicidal Ideation groups. IL-6 and TNF-alpha levels have been previously reported elsewhere (Lindqvist et al., 2017).

	MDD (*n*=48)	Control (*n*=54)	P-value	MDD with Suicidal Ideation (*n*=17)	MDD without Suicidal Ideation (*n*=31)	P-value
Age (years. mean ± SD)	39.3 ± 14.9	37.9 ± 13.9	0.71	42.1 ± 14.3	37.7 ± 15.2	0.27
Sex (% female)	56%	61%	0.62	53%	58%	0.73
BMI (mean ± SD)	26.0 ± 4.5	24.5 ± 4.9	0.08	25.5 ± 4.2	26.3 ± 4.7	0.61
Ethnicity (%) Caucasian Black/African-American All other	 52% 8% 40%	 57% 6% 37%	0.80	 53 0% 47%	 51.5% 13% 35.5%	0.28
Smoking (% Smokers)	27%	6%	<0.01 **	24%	29%	0.68
Sampling season (% summertime)	48%	50%	0.83	29%	58%	0.06
HDRS total score (mean ± SD)	20.3 ± 33.1	N/A	N/A	22.1 ± 4.25	19.3 ± 2.18	<0.01**
25(OH)D, nmol/L (mean ± SD) *a)*	51.1 ± 20.0	54.9 ± 21.0	0.24	48.4 ± 19.0	52.6 ± 20.7	0.61
NLR (mean ± SD) *a)*	2.2 ± 0.9	2.3 ± 1.2	0.98	2.3 ± 1.0	2.2 ± 0.8	0.81
WBC (mean ± SD) *a)*	5.5 ± 1.4	5.6 ± 1.5	0.58	5.5 ± 1.8	5.4 ± 1.2	0.96

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

*a)*25(OH)D, NLR and WBC were normalized using 90% winsorization prior to parametric analyses. P-values are based on these calculations but raw, non-winsorized, means are given here.

Table 2.

Correlations between 25(OH)D and different variables in all subjects and MDD divided into Suicidal Ideation/ Non-Suicidal Ideation groups, Pearson's r.

Variable	All subjects (*n* = 102)	MDD (*n* = 48)	Control (*n* = 54)	Suicidal Ideation MDD(*n* = 17)	Non-Suicidal Ideation MDD (*n* = 31)
Inflammation Composite Score	r=−0.30 (p<0.01) **	r=−0.34 (p<0.05) *	r=−0.22 (p=0.11)	r=−0.56 (p<0.05) *	r=−0.20 (p=0.30)
NLR [a]	r=−0.10 (p=0.34)	r=−0.37 (p=0.01) **	r=0.08 (p=0.57)	r=−0.71 (p<0.01) **	r=−0.16 (p=0.39)
WBC [b]	r=−0.15 (p=0.14)	r=−0.37 (p=0.01) **	r=0.00 (p=0.99)	r=−0.52 (p<0.05) *	r=−0.26 (p=0.16)

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

[a]Missing data from one MDD and two controls

[b]Missing data from one control